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Field Studies

ON THE

Dissemination and Growth

OF THE

Chestnut Blight Fungus

BY

PAUL J. ANDERSON AND D. C. BABCOCK

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Pennsylvania Chestnut Tree Blight Commission.

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NOTE.

The Commission for the Investigation and Control of the Chestnut Tree Blight Disease in Pennsylvania was authorized by an Act of Assembly approved by Governor Tener, June 14, 1911.

This Commission, in collaboration with the Pennsylvania Department of Forestry, is to ascertain, determine upon and adopt the most efficient and practicable means for the prevention, control and eradication of a disease of the chestnut tree, commonly known as the chestnut tree blight. It is authorized to conduct scientific investigations into the nature and cause of such disease, and the means of preventing its introduction, continuance and further spread. The Commission has power to establish, regulate, maintain and enforce quarantine against the introduction and spread of such disease, and from time to time, to adopt and prescribe such regulations and methods of procedure as it may deem necessary and proper.

The Commission will cooperate with the owners of chestnut trees to accomplish all the purposes of the Act in every possible manner.

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INTRODUCTORY STATEMENT.

The work reported in this Bulletin was carried out under the direction of the Field Pathologist in two field laboratories of the Pennsylvania Chestnut Tree Blight Commission during the growing season of 1912. One of these laboratories was located at Charter Oak, Huntingdon County; the other was at Mt. Gretna, Lebanon, County.

The writers wish to acknowledge valuable assistance from the following: E. T. Kirk, J. F. Burrows, M. R. Clare, C. F. Korstian, L. S. Pearson, A. B. Bechtel, W. E. Keefer, R. D. Spencer, C. A. Gates.

DISSEMINATION OF THE FUNGUS

INTRODUCTION.

Not only must the chestnut blight fungus be destroyed where it is already found, but it must be prevented from spreading to healthy trees. The loss of the trees already infected would be a small matter if we had a way to prevent it from spreading to those that are now free. But this way will be found only after it has been determined how the fungus passes from one tree to another, and how it gains entrance to a healthy one. This is the fundamental problem and no small part of the summer's work has been directed towards its solution. The work is far from complete but a report is submitted at this time for the benefit of others, who may be working along this line.

Before entering into a discussion of experiments, a resume will be given of what others have said and done on the problem of dissemination.

A REVIEW OF THE LITERATURE.

How the fungus enters the host.—Murrill (1) in 1906 is of the opinion that the fungus could only enter through wounds but suggests the possibility of lenticels also being channels of entrance. He thinks that wounds may be made by any one of a number of agents: mice, voles, rabbits, man, insects, etc. Later in the same year (2) he believes that the fungus may enter through dead twigs, since he finds these at the center of many cankers. Metcalf, (3) in 1908 says that the spores enter through wounds, insect punctures, dead twigs or dead wood. Later in the same year Hodson (4) repeats the statements of Murrill and Metcalf. The next year Metcalf and Collins (5) state that the spores enter through wounds and possibly in other ways. In 1910, however, they (7) assert that the fungus can enter without any visible break in the bark. Still they are of the opinion that wounds are the usual channel and state that among these, the tunnels of the bark borers are the most common. They suggest also that winter injury may produce lesions that will give entrance to the fungus. The idea of the borers being responsible was evidently strengthened by further observation for in 1911 (9) after repeating their former statement, they write, "In many parts of the country where the disease is prevalent there is very direct evidence that bark borers, and particularly the two-lined chestnut borer (Agrilus bilineatus) are directly associated in this way with 90 per cent. or more of all cases of this disease." None of these writers cited give any experimental data to prove their assertions.

How the fungus is carried from one tree to another.—Whether the agent that makes the wound is the same as the one that carries the spores, is a question on which observers do not agree. The tendency in the last few years has been toward the belief that they are the same, and that when the specific agent that makes the wound is found it will also be found to carry the spores.

Murrill (1) in 1906 says that the summer spores are disseminated by wind, insects, birds, squirrels, etc., and also that mice, voles and rabbits make wounds and carry the spores in their fur. In a second article (2), he suggests the agency of rain in carrying the spores to other parts of the tree. Hodson (4) in 1908 says, "Wind is probably the principal agency, but the spores are no doubt carried by animals, birds, insects and by the shipment of infected material. The disease spreads locally through the gradual distribution of the spores from tree to tree and at a distance, chiefly through the shipment of infected material, such as a nursery stock, bark, nuts and other products. There is a possibility that long distance infection is also effected by means of migratory birds." It should be noticed that he was speaking only of the summer spores. He does not claim to have done any work on this subject himself, but has compiled his circular mostly from what Metcalf and Murrill wrote. Rane (8) in 1911 says that the spores are carried long distances by the wind but it is impossible to tell whether he was speaking of the conidia or winter spores. Exactly the opposite opinion is expressed by Metcalf and Collins regarding the spores (9): "There is no evidence that they are transmitted by wind, except where they may be washed down into the dust and so blown about with the dust." The following year Metcalf stated as follows:" Both kinds of spores appear to be sticky and there is little evidence that they are transmitted to any distance by wind except when washed down into the dust and so blown about with it." (10). To account for the spread they suggest rain and add: "There is strong evidence that the spores are spread extensively by birds, especially woodpeckers, and there is also excellent evidence that they are spread by insects and by various rodents, such as squirrels." (9). They also mention the agency of man by shipping timber and nursery stock. The general opinion of investigators up until the present about the wind, is well expressed by Collins* who in his address at Ithaca, N. Y. in December 1911, makes the following statement: "I am quite convinced that these spores are not blown broadcast, simply because they are of a sticky nature." He adds that there is no reason why birds should not carry them.

As far as any published research is concerned, the situation was well summed up by Rankin (11) at the Harrisburg Conference in February, 1912: "Concerning the means of the spread of fungus from one tree to another, we have nothing except secondary evidence. Most writers have theorized on the different methods by which the conidia or summer spores might be carried from one tree to another and new infection started. Reasoning by analogy with what is known of the behavior of many fungi, such agencies as borers, birds, ants and the wind, etc., have been suggested but in no wise proved to be responsible. It seems that the ascospore stage has not been considered by any writer in the dissemination of the fungus, yet this stage follows the conidia very quickly and is the more abundant fruiting stage." Then he adds: "Under moist conditions, the ascospores are shot forcibly out in the air, where they can be caught up by the wind and carried for a considerable distance. The speaker found the ascospores being shot from the mature pustules during every rainy period last summer. The question at once arises, why could not these ascospores once shot into the air, be carried long distances and owing to their abundance cause a large majority of the infection?" So far as the literature shows, Mr. Rankin stands alone in his views of the importance of the ascosporic stage and the agency of the wind.

Fulton (11) reports some work carried out by Mr. R. A. Waldron, which has an important bearing on dissemination. Attempts were made by a strong blast from an electric fan to blow the conidia into the air. The results were such as to lead Mr. Waldron to believe that at best the conidia could be blown only a short distance even in a strong wind. His opinion, expressed to the writer, is that the wind has very little to do with the dissemination of the conidia.

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*Collins, J. F., The Chestnut Bark Disease. Reprint from Proceedings of the Second Annual Meeting of the Northern Nut Growers' Association, Ithaca, N. Y., December, 1911.

CHANNELS OF ENTRANCE TO THE HOST.

Much confusion has arisen in the past by not making a distinction between the agent that carries the spores, and the one that produces the wound by which the spores may enter. Some writers have proceeded on the assumption that one and the same agent is responsible for both, i. e., that this agent carries the spores to a healthy tree and there makes the wounds where it deposits the spores in a favorable place for growth. That such is not the case, can positively be demonstrated in many cases and the evidence is strongly against it in the majority of cases.

Necessity of a wound.—Murrill (1) failed to get an infection except where a wound was first made. Metcalf and Collins (7) however, state that the parasite may enter without a visible abrasion in the bark. In our experimental plots all attempts to get an infection by placing the spores on sound bark have failed. The following experiment, however, may be not without significance in this respect. Diseased bark taken from a young canker was placed closely around small branches, where no abrasions could be detected and then the whole wrapped with cotton. The cotton served first to exclude insects and other agents; second, to keep the bark moist. Seven branches were treated in this way on June 29th and on September 5th,—a little less than ten weeks, cankers were formed on three of them. There was no sign of an infection, however, one month after the experiment was started. On account of the unusually favorable conditions for infection that are offered and the tardy appearance of the cankers, not much practical importance has been attached to these results. All other experiments indicate that the cases where the fungus enters through sound bark are so rare as to be entirely negligible.

The value of observation of natural infections.—The questions that have been asked hundreds of times are: Can't you tell by looking at young cankers, how they were started? What is the use of making inoculations when you have the wounds right there at the center to show you where the canker started? The problem is apparently very simple. If you find a canker with a larval gallery at the center, then the fungus must have entered through that wound;

if a sapsucker hole is there, then it is plain that the infecting spore was deposited in that hole, etc., etc. It is safe to say that ninety-nine per cent. of the statements that have been made concerning the infecting agent are based on data collected in this way. Some extensive lists of the kinds of wounds in the canker have been carefully compiled and put forward as indicating that certain specific agents are responsible for a certain percentage or the infections. The fallacies of such data are so obvious that they hardly need comment. It is almost impossible to tell, except in the very youngest cankers, whether the wound preceded the canker or the canker preceded the wound. Very few old cankers are free from larvae and it is not uncommon to find them in cankers less than an inch in diameter in our experiment plots and yet the wounds were produced by a knife and not by the larvae. Woodpeckers and other birds pick at the cankers to get the larvae. The holes that they make, often lead the uninitiated to believe that the canker started in them. Many have stated that the natural cracks in the bark are a favorite means of entrance because they found these at the center of the canker. Yet it has been noticed all summer, that it does not matter by what method the inoculation is made,—a crack will almost invariably be formed at the center on account of the drying out of the bark and soon it is almost impossible to tell by what method the inoculation was made. In our observations on natural cankers, dead twigs have more often been observed at the center than any particular wound. But did the canker start from the dead twig or did the twig die as a result of the canker forming around it? Very young cankers are not often noticed and when they are, they usually contain no spores, so that it is impossible to state whether or not they were produced by Endothia unless cultures are made or they are put in a moist chamber for further development. Many cankers have been ascribed to this fungus, but when sent in to our laboratories for examination proved to be only the natural dying of the bark around insect galleries.

Observations on the natural cankers are not altogether without value, but it is certain that data collected from these observations alone are not reliable in determining the cause of infection.

Inoculations of various kinds of wounds.—The only way then to find out what agents are responsible for giving entrance to the fungus is to find the wounds produced by these agents (in uninfested territory), inoculate them artificially and see if cankers are developed from them, at the same time keeping plenty of wounds not inoculated as checks. In some cases where wounds cannot be found where wanted, it is necessary to make artificial wounds as near like the natural ones as possible. The following kinds of wounds have been inoculated and the results given in Table 1.

1. Slits in the bark, longitudinal, diagonal, etc., to imitate axe wounds, knife wounds, etc.
2. Gouges to imitate climbers.
3. Artificial borer holes.
4. Natural insect holes.
5. Peeling down the bark.
6. Scraping off only the outer cork layer.
7. Cut stubs.
8. Broken down branches.
9. Natural cracks.
10. Gimlet holes to imitate sapsucker holes.
11. Holes made by a hypodermic needle.

It will be noticed in this table that a fair percentage of infection was secured in all but three kinds of wounds. One of these was natural cracks. Indications from later experiments, not yet reported, lead us to believe that this kind of a wound also can become infected. It is worthy of remark that out of all the natural insect holes that have been inoculated with both kinds of spores and with cultures, not one has produced a canker up to date.

The results of all the inoculation experiments of the summer, certainly warrant the following conclusion: Any kind of a wound in the bark deeper than the outer green cortex may furnish an entrance for the fungus. In other words, it is not necessary to have a wound of any specific character or made by any specific agent.

Before leaving this subject, mention should be made of another mode of entrance, which although as yet not sufficiently investigated, may prove to be of some importance. It was commonly noticed in thick young coppice in eastern Pennsylvania that many of the young sprouts of this year's growth were dying from the tip downward. The disease seemed to start in the leaves, the midrib especially being blackened by the invasion of a fungus. All stages could be found from leaves with dead tips to the entirely deadened twig. In a very large number of cases the blight fungus was growing around the base of the twig, where the blackening had run down to the larger branch, in such a manner as to lead one to believe that it had entered by means of the twig. Isolations both from the leaves and from the twigs showed the presence of a fungus which we have repeatedly demonstrated to be the cause of "die back" on the twigs in western Pennsylvania. This fungus was isolated too late in the season to carry on inoculation experiments this year. That its attack may have some relation to the entrance of the blight fungus is at least possible.

TABLE 1

Showing the comparative value of different kinds of wounds for infection.

Character of wound.	Inoculation material used.	Number of inoculations.	Percentage successful.
Longitudinal slit,	Diseased bark,	568	93.6
Longitudinal slit,	Mycelium from culture,	454	89.2
Diagonal slit,	Mycelium from culture,	25	96.0
Diagonal slit,	Dry spore horns,	16	93.7
V-shaped cuts,	Conidia in water,	97	93.8
V-shaped cuts,	Ascospores in water,	88	94.3
V-shaped cuts,	Ascospores shot in dry,	1238	82.5
Artificial borer holes,	Mycelium from culture,	68	54.4
Natural insect holes,	Mycelium from culture,	18	0.0
Natural insect holes,	Conidia in water,	23	0.0
Natural insect holes,	Ascospores in water,	22	0.0
Peeling down bark,	Mycelium from culture,	25	88.0
Stab with knife	Ascospores in water,	347	36.9
Stab with knife	Conidia in water,	81	86.8
Stab with knife	Dry spore horns,	96	79.1
Scraping off outer cork layer,	Mycelium from culture,	25	0.0
Cut stubs,	Mycelium from culture,	22	81.8
Broken branches,	Mycelium from culture,	45	71.9
Natural cracks,	Mycelium from culture,	25	0.0
Gimlet holes,	Ascospores in water,	135	52.6
Hypodermic needle,	Ascospores in water,	54	75.9

Having thus disposed of the agents which furnish a means of entrance to the new host, let us next consider some of the agents, which have been suspected of carrying the disease from tree to tree.

MAN AS THE DISSEMINATOR.

The shipment of nursery stock.—This has proved to be an efficient means of carrying the fungus by long jumps to regions free from blight (Cf. (5) p. 49; (4) pp. 5 & 7.) This phase no longer calls for experimental proof, but four interesting cases which have come to light in western Pennsylvania are worthy of mention because they were all far beyond the main line of advance of the disease. The first of these was near Connellsville, Fayette County, a county in which blight has never been found on the native trees. Twelve

Paragon trees had been purchased from a Philadelphia nursery in the Spring of 1911. These were grafted trees about two or three years old. When the disease was discovered about a year afterward, six of the trees were dead or in a dying condition from cankers on both the stocks and the scions. Fortunately no native trees were close to these and the fungus had no chance to spread further. A second case at Warren was similiar. Out of twelve trees, eleven were dead at the top and on the majority of the trees Endothia was plainly the cause of death. A very similar condition was found in Elk county. Here however, it had gone further and one native tree close by was badly infested. These trees were brought from Rochester, New York. The fourth case is between Somerset and Berlin in Somerset County, and here it has been left long enough to demonstrate the awful destructiveness of the disease. About four years ago some Paragon grafts were brought from Lancaster County, a badly infested county, and top grafted on native trees. There are now thousands of diseased trees within a radius of two miles from the grafted trees. Since the disease has gone so far and since there were no records of it up to the present year, it is only fair to state that it cannot be definitely proved now that this infection started from the Paragon grafts. The fact that the scions were from an infested orchard indicate that these were the source of infection.

The spread of the disease by tools.—To determine whether the disease can be spread by tools in cutting into a diseased tree and then into a healthy one, the following experiment was tried:

On July 25th, 13 cuts were made in trees with an axe, each time after chopping into a diseased log several times. Within six weeks cankers began to appear around the cuts and on October 1st, when the trees were cut down and burned, 12 of the 13 cuts had decided cankers about them. There is then little doubt that the disease can be carried in this way.

Shipment of logs and wood.—Can the disease be spread by shipping logs and wood into uninfested territory? This resolves itself mainly into the question of whether the fungus lives and grows and produces spores on the dead bark and logs, under the conditions in which they are usually kept. How long will it live there? Will it pass from one log to another or from one piece of bark to another? Supposing that the spores were already formed, how long would they continue to live on dead logs or on bark? How long would the perithecia retain their power to shoot spores into the air? To answer these questions, the following experiments have been started and the results up to date are given below:

Experiment: To determine how long mycelium will remain alive in logs, peeled and not peeled. On July 1st, 104 logs were cut and left on the ground where they fell; 18 of these were peeled and the remainder left with the bark on them. Three months afterward the mycelium was found still alive in 22 per cent. of the peeled logs and 66 per cent. of those not peeled.

Experiment: To determine whether bark after being taken from the log and thrown on the ground can become infected. Pieces of bark were inoculated and thrown on the ground in various situations to see if the fungus would develop on them. Table II gives results.

TABLE II.

Showing results of inoculation in removed bark.

Date.	Method of making inoculations.	Where kept.	No. of incubators.	Percentage successful.
June 1,	Mycelium from culture in slit,	Dry ground where sun was shining.	14	0
June 1,	Conidia put in slit in bark,	Dry ground where sun was shining.	12	0
June 1,	Ascospores put in slit in bark,	Dry ground where sun was shining.	15	0
July 23,	Mycelium from culture in slit,	Shady place but rather dry ground.	6	100
July 23.	Ascospores in water,	Shady place but rather dry ground.	3	100
July 23,	Piece of diseased bark tied on,	Shady place but rather dry ground.	3	100
Aug. 21,	Piece of diseased bark tied on,	Low wet ground in shade,	12	100
Aug. 21,	Washed with water containing ascospores.	Low wet ground in shade.	7	100
Aug. 21,	Mycelium from culture in slit,	Low wet ground in shade.	21	85

From this table it appears that the condition under which the bark is kept will determine whether or not it can become infested. If piled in a moist and shaded place, it is certain that the fungus will spread through it, if any spores are present, to infect it. Also if diseased and healthy bark are piled together, the fungus will run from the diseased bark to the bark that is uninfested. It has often been noticed during the past summer that where diseased trees were cut and the chips left in a pile about the base of the tree, that the fungus will grow luxuriantly in the bottom of the pile. Not only will it grow on the dead chips, but also on leaves and dead chestnut burs, as proved by the following experiments:

Experiment: To determine whether Endothia will grow on dead leaves. Dead chestnut leaves were sterilized in a moist chamber on July 23rd and then a few drops of water containing ascospores washed down over them. On August 24th numerous pycnidia were formed on the leaves.

Experiment: To determine whether Endothia will grow on dead chestnut burs. On June 14th, three dead chestnut burs were sterilized and placed under a bell jar. Afterwards a few drops of water containing ascospores were washed down over them. On July 24th pycnidia had been developed on all of them.

Experiment: To determine whether the fungus can grow on seasoned chestnut wood. On September 13th, nine small pieces of a rail that had been seasoned for several years, were put in tests tubes with moist cotton, sterilized and inoculated at one end. On October 6th, scattered pycnidia had formed at various points on the surface of all of them. That it can also grow on twigs of other species will be brought out later.

The fact that this fungus, besides being a virulent parasite, is also an excellent saprophyte seems never to have received sufficient attention. It will grow more rapidly through dead tissue than through living tissue, and will live there for a long time and continue to produce its spores. Some interesting examples of this have been noticed. In an infested tract previously mentioned, in Somerset County, in June of this year, diseased trees were burned so near healthy ones, that the latter were scorched on one side and the bark cracked open. A reinspection of the injured trees four months later showed that the fungus had gained entrance through the cracks and had spread entirely over the burned sides of the trees, growing in some cases a distance of six inches from the point of infection. Comparisons with the rate of growth as determined by inoculation experiments show that this is a great deal faster than it grows through healthy tissue. At St. Marys, Pennsylvania, trees with scattered cankers were cut in the spring of this year and permitted to lie without further attention. In October a reinspection showed the fruiting pustules of the fungus spread more than a foot from the edge of the canker during the summer. Trees in the same condition were felled during the spring at Anderson, Pennsylvania. The trunks were utilized but the tops and branches were left on the ground. Six months later, hardly a branch or stump or top could be found which was not fairly covered by the fungus. In the saprophytic condition no canker is formed and the bark looks no different from ordinary dead bark except for the reddish pustules. Another peculiarity of the fungus under these conditions is that the mycelium does not advance in fan-shaped mats, but by single strands so that it is not readily visible to the unpracticed eye.

Spores that are already formed live for months (see below under "Longevity of the Spores") and that the perithecia will still retain their power of ejecting the ascospores into the air is proved by the following:

Experiment: To see how long after being dried the perithecia can eject the spores. Bark with perithecial stromata was kept in the laboratory for 14 weeks. Five pieces of this were then tested and ascospores were shot out on slides from two of them.

Then there is no reason why the disease can not start in a new locality to which the logs are shipped, if in that locality they are placed near where chestnut trees are growing. If the logs are peeled, however, the chances of spreading are much reduced since the logs will dry out more and in any case only pycnidia will be produced. The shipment of unpeeled wood is evidently a more prolific way of spreading the disease since the perithecia are developed on the bark.

The only recommendations about the shipment of chestnut products that can be made at this time are in regard to the moisture conditions under which they are shipped or stored. It has been demonstrated that water is necessary first for the ejection of the ascospores; and second for the germination of either form of spores. Shipment should be made in closed cars and the wood, if it cannot be stored inside, should at least be piled up off the ground in such a way as to admit all the air and sunlight possible.

BIRDS AS CARRIERS OF THE FUNGUS.

The fact that birds pick at the cankers in search of larvae has been previously mentioned. Then they would get the spores or bits of mycelium on the feet, bills and feathers and carry them away to other trees and deposit them there seems a plausible theory. During the early spring, on the experimental plats at Mt. Gretna, Mr. Clare observed several species of woodpeckers and the blue jays picking at these cankers, and in some cases large areas were picked away. At that time, however, none of these were shot and tested for the presence of spores. A set of experiments was planned to determine whether any of the birds carried the spores, but on account of unfortunate delays, could not be carried out until the middle of the summer. By that time the birds were very scarce around the trees. It seems that during the summer they live mostly on berries and other fruits and do not pick at the trees. Nevertheless some of them were shot in the plots and tested. The method and results are given below:

Birds found on the infested trees were shot during the summer and their feet, bills, and tail feathers washed separately in sterile water. This water was then centrifuged to bring down the spores that might have been washed from the birds. Part of the sediment was then examined under the microscope and the other part plated out in dilution plates. When colonies of fungi appeared, they were isolated to determine whether they were Endothia. Another method used, was to make direct imprints on sterile chestnut bark agar plates with the feet and bills of the birds. Three blue jays, eight downy woodpeckers, three creepers, four flickers and two hairy woodpeckers were treated as above, but all results were negative. To determine whether this method was at fault, the feet and bills of birds were brought in contact with both conidiospores and ascospores and then treated as above. Colonies of Endothia developed in abundance.

Birds may be instrumental in spreading the disease but up to the present we have no experimental data to prove it. These experiments will be resumed during the winter and spring with a better chance of obtaining conclusive results.

That such a wound in the bark as that produced by a sapsucker could become infected by ascospores is indicated by the following:

Experiment: One hundred and thirty-five wounds of about the same diameter and depth as the holes made by sapsuckers were made with a gimlet. These were inoculated by putting the ascospores in water and dropping the water from a pipette into the holes. All of these had cankers formed about them six weeks later. No cankers appeared on the forty-five uninoculated gimlet holes used as checks.

HOW THE RAIN SPREADS THE DISEASE.

The rain dissolves the mucilaginous matrix of the spore horns and the conidia are carried down the trunk, where they probably find lodgment in wounds and produce cankers. This is the usual explanation of the fact, that most trees with cankers on the upper trunk or large limbs, later become diseased at the base and on the exposed roots. As an actual fact, there are no experimental data which prove that the rain is responsible for these basal cankers. Insects might just as well carry the spores there, and several other agents might be suggested but the rain theory is the most plausible

Some very interesting data were collected along this line at Charter Oak on the naturally infested tract. The diseased trees were blazed April 1st of this year, the blaze being cut in healthy bark in most cases. On August 14th eighty-four of the blazes were examined and sixty-six of them had developed new cankers at the base of the blaze, while only eight had cankers at the top. Now a remarkable thing about the Charter Oak infection is that all through the summer no ascospores could be found, but there has been an abundance of conidial tendrils since the middle of May. Taking these facts into consideration, therefore it seems probable that the cankers in the blazes were started by the conidia washed down from above.

To determine that the spore horns are washed off by the rain, it is only necessary to watch the water running down the trunk during a rain. That they would wash into wounds below is certain, if there were wounds there at the time of the rain. Then to duplicate these conditions, it is only necessary to make a suspension of conidia in water and spray trees so that the conidia run down into wounds, or to put the water with the spores directly in the wound. This was done successfully in various sorts of wounds as reported in Table III.

A still more convincing experiment was carried out at Mt. Gretna as follows:

Experiment: Isolated trees were selected which had cankers on the trunks producing conidial tendrils, but having no ascospores in them. Wounds were made at various distances below the cankers. Water was sprayed with an atomizer on the cankers and allowed to run down the trunks into the wounds. Of the twenty-three wounds treated in this way, sixteen developed cankers later.

TABLE III.

Showing the value of conidia in water for producing infection.

Date.	Plot.	Method of inoculation.	Number of inoculations.	Percentage successful.
June 5,............	6	Sprayed with atomizer in V-shaped cuts,........................	57	89.4
June 17,............	15	Dropped into stab in the bark,..	53	54.7
June 11,............	12	Dropped into stab in the bark,..	96	55.00
July 7,............	36	Dropped into V-shaped cut,..	40	92.5

A much more important part played by the rain in the spread of the disease is in soaking up the bark and the perithecial stromata, thus bringing about the proper conditions for the ejection of the ascospores. This will be treated under "Wind Dissemination."

Sometimes it has been observed that the ascospores, instead of shooting, merely ooze out, and in this case, the rain would wash them down and produce basal infection, just as with the conidia. To determine the power of ascospores to produce infection when carried down by the drops of rain water, a set of experiments was carried out very similar to those with conidia. The rain might also splash the spores for short distances or carry them to trees that are directly under cankers on the higher trees. The results are given in Table IV. On the whole there is need of further experiment in regard to the relation of the rain to the disease.

TABLE IV.

Showing the value of ascospores in water for producing infection.

Date.	Plot.	Method of inoculations.	Number of inoculations.	Percentage successful.
June 10,............	10	Dropped into stabs in bark,..	184	29.3
June 11,............	13	Dropped into stabs in bark,..	144	34.7
June 17,............	15	Dropped into stabs in bark,..	59	40.7
July 17,............	36	Dropped into V-Shaped cuts,..	88	88.9

THE RELATION OF INSECTS TO THE DISEASE.

From the time that the disease was discovered, insects have come in for a large share of the blame for its spread. The main reason for this theory seems to be that they are found so abundantly on and in the bark of chestnut trees and that their galleries are common in the cankered areas. But one will look in vain through the literature for any convincing experimental data to prove that they are responsible. Since this was considered a problem for the entomologist, and since there were several entomologists working on it in Pennsylvania, very little work bearing on the relation of insects was done in our laboratories.

The insect most often found in this state, working in the bark is the little larva of the "bast miner." When it emerges it leaves a neat little hole less than 0.5 mm. in diameter. Since these appeared to be excellent places for the fungus to gain an entrance, inoculations were made in them, with mycelium, ascospores and conidia and introducing them in various ways and at different times during the summer. No infection was ever secured.

There seems to be a general idea abroad that insects are in the habit of boring into diseased bark and then going to another place, and boring another hole there, thus carrying the disease from one tree to the next. Competent entomologists assure us, however, that it is doubtful if any insects with such habits live on the chestnut. Larvae do not leave their galleries until they come out as adults, and then they come out leaving the old pupal case behind them, or else they come out and go into the ground to pupate, in which case also they would not carry the spores to other trees. There is also an idea current that when the adults deposit their eggs they sting or puncture the bark and lay the eggs on the inside. Now as an actual fact we are informed that such is not the case but that the eggs, as a rule, are deposited on the outside of the bark, and when the larvae hatch, then enter the bark through microscopically small holes and at so slow a rate, that it is doubtful if very much fresh bark is left exposed at any one time. The cicada is an exception to this rule, since it does deposit its eggs on the inside and make a large wound in doing so, but has never been demonstrated that a cicada will oviposit in a diseased area, so that it would be hard to see how the spores could get on the ovipositor in the first place. Nor are the cicadas sufficiently numerous in this part of the State to account for the infection. The writers have had occasion to examine several thousand cankers during the summer, but have never seen one that was suspected of having started from a cicada wound. However, cases have been reported by field men in which as high as thirty per cent. of the lesions were found in or about cicada wounds.

Ants have been accused and some observers state that they have actually seen them eat the spore horns and pustules and also carry them about with them. Even at that, this is only secondary evidence that they produce new infections with these spores.

Experiment: On May 8th, eighteen ants were dug out of a canker and each transferred to a sterile plate of potato agar and permitted to run over the plate for several days. No colonies of Endothia developed on the plates. This experiment was duplicated later by R. D. Spencer in the laboratories at Charter Oak, but with negative results. Mr. Spencer is of the opinion, however, that this method is at fault.

Experiment: On August 7th, five vials of insects were sent to Charter Oak from eastern Pennsylvania by P. H. Hertzog, to be tested to see if they had spores of the fungus on them. These were tested by putting them, one at a time, in tubes or melted agar, kept just warm enough so that it would not solidify. The tube was then shaken and the agar poured into sterile Petri dishes. Ants from three of the vials proved to have the spores on them. The spores had been artificially placed on the ants in one of the vials, however. The ants in the other two had been taken from the bark of the diseased chestnut trees after a rain. This indicates that the ants can carry the spores.

Experiment: To find out how long spores will remain on ants. On August 28th, fifteen large ants were caught and immersed in water, which was milky with conidia. Then these were allowed to run in a bottle of earth and at regular intervals two were taken out and tested for the presence of spores. The last test was five hours and sixteen minutes afterward and spores were still on the insects. The technique used was the same as in the preceding experiment. This shows that ants may retain the spores long enough to carry them some distance.

Experiment: To determine whether ants eat the stromata of the fungus. On August 28th, fifteen ants were placed in a bottle, containing moist bark with perithecial stromata. Air was admitted through cheesecloth at the top. They were kept in there until all but two of them starved to death. Examination showed that they had not eaten the pustules. No similar experiment with spore horns has been tried.

Many observers have noted the fact that the stromata at times are found to be all eaten out of the bark. This has been attributed to various agents, such as birds, squirrels, ants, etc. This has been especially noticed during the past summer and trees have been found covered with cankers, but with not a pustule remaining. It was thought that the agent that removed the stromata might be responsible for spreading the disease, by carrying the spores to healthy trees. Mr. Spencer worked on this problem and found that although several insects occasionally work at the pustules, by far the greater part of them in this locality, were eaten out by *Leptostylus maculata*,—one of the Cerambycid beetles. These insects were repeatedly put in cages, with pieces of the bark containing stromata, and it was a matter of only a few days when not a pustule remained on the bark.

The question next to be answered was, whether the disease was further disseminated by the ravages of this insect or whether the beetle was beneficial since it ate such a large number of spores.

Are the spores digested and thus destroyed in the stomach of the insect or do they pass out in the excreta to germinate on the other hosts? Two sets of experiments were run to determine this point.

Experiment: The beetles were fed on the stromata for five days then taken out and sterilized with phenol on the outside. Then the viscera were removed with sterile needles, put in melted potato agar and plated out by the usual poured plate method. Numerous colonies of bacteria developed but no fungi at all. The bacterial colonies are explained by the fact that bacteria thrive in the intestines of insects, as well as higher animals. This experiment was checked by the following:

Experiment: After being fed for a day on the ascosporic stromata five of these insects were removed and caused to excrete the fecal material by a light pressure with the forceps on the abdomen. This fecal material was caught in sterile potato agar tubes and plated out with the same negative results as in the preceding experiment. These experiments indicate that this insect may be really beneficial.

Mr. Spencer and the writers are of the opinion that the insects are not important agents in the spread of the blight, except in so far as they produce wounds by which the spores may enter.

THE RELATION OF THE WIND TO THE SPREAD OF THE CHESTNUT BLIGHT.

Murrill in his first publication on the blight (1) in June, 1906, states that the summer spores are disseminated by the wind. Outside of mentioning the fact that the winter spores are matured in late autumn, this stage of the fungus is entirely ignored. From that time until 1911 almost every writer on the chestnut blight who mentions dissemination at all, follows Murrill in stating that the conidia are blown by the wind and in ignoring the ascospore stage. The disease does spread in a way that would lead most observers to suspect that the spores are carried by the wind; therefore their conclusions are not remarkable. It is much easier to imagine the wind blowing the spores off these exposed tendrils of the summer spores, than out of the perithecia, which are deeply imbedded in the stromata. Besides, the ascospores were thought to be developed in the winter, while the disease spreads most in the summer. But when it was discovered that the conidia are very sticky when wet, and are cemented together in a mass as hard as horn when dry, the wind dissemination idea had to be discarded. The general opinion concerning conidia as expressed by Metcalf and Collins (9) in October, 1911, was as follows: "As both kinds of spores are sticky, there is no evidence that they are transmitted by wind except where they may be washed down into the dust and so blown about with the dust."

The results of a series of experiments during the last summer, have led us to believe that the ascospore stage is the important stage in the dissemination of the fungus and that the wind is largely responsible for its spread. The observations and data upon which these conclusions are based are given below.

Occurrence of the ascospore stage.—The ascospores are commonly called winter spores but this name is misleading. There has never been a time during the past summer when ascospores could not be found maturing in any number of localities in Pennsylvania. On the other hand there were "spot infections" in the western part of the State, where nothing but the summer stage could be found, although the infection apparently was of several years standing. A comparison of a large number of these "spot infections" showed that in general, where the fungus was all in the summer spore stage, young cankers were scarce and were mostly confined to the young growth about the older infested trees, while in the localities where the winter stage was common, they were more numerous and much more widely spread in the surrounding woodland. These were the first observations that led us to believe that the ascospores are of primary importance in spreading the disease from one tree to another.

Ejection of the ascospores.—These spores are enclosed in a tough leathery flask, the perithecium, which in turn is deeply imbedded in the stroma. In order to get to another tree and to reproduce the disease there, they must be removed from this flask and get out into the air. The question at once arises: How do they get out? In the summer of 1911, W. H. Rankin (11) discovered that the spores are forcibly ejected from the ostioles of the perithecia. That such is the case can very easily be demonstrated. After a heavy rain or after making the bark very wet by spraying water on it, fasten a glass side on the bark over mature perithecia, so that the surface of the slide is only a few fillimeters from the ostioles. In a short time white blotches will appear on the slide over certain of the ostioles which are active. Examination under a microscope will show these to be little heaps of ascospores sticking to the slide. The course of the spores after leaving the ostioles can be watched under the low power of the microscope or better under a binocular dissecting microscope. The writer has also often observed them with only a hand lens. A still better method of watching them in quantity and one which has been used successfully in our laboratories is the "light-beam" method, which is described by Buller in his "Researches on Fungi." By this method they can be watched with the naked eye, shooting out into the air by thousands.

The relation of the rain periods to the ejection of spores.—The spores are ejected only during periods of rain since the bark must be well soaked. To see how often they would shoot during the month of August under natural conditions, slides were suspended over 30 groups of pustules on a clump of trees. All of them ejected spores, at least once during the month, and four of them at five different dates; others, less often. The fact was noticed that even after a heavy rain, the spores would often be ejected only from the stromata on one side of the tree, the other side not being sufficiently drenched to start the perithecia.

Time required for perithecia to begin shooting spores after the bark is soaked.—This, of course will vary with the moisture content of the bark before the soaking begins. To make conditions uniform, specimens of bark which had been dried for three weeks in the laboratory were used in a number of experiments. They were drenched, then put in the bottom of Petri dishes, into which had been poured a little water to keep the bark continually moist. In all, 35 pieces of bark were used. Of these 22 had shot spores within two hours; 5 began shooting in 45 minutes. The average time for the 22 was 1 hour and 28 minutes. In another set of experiments, fresh bark was brought in from the woods and tested. The variation was greater due to the different conditions under which the bark was used. Several specimens ejected the spores in less than three minutes after they were brought into the laboratory.

The duration of the shooting period following a rain.—The following experiment gives data both for answering this question and also additional data on the one just discussed: Sixty pieces of ascospore bark were soaked for 15 minutes and then slides suspended over them to detect the spores that were shot. Ninety per cent. of them ejected spores: The first one started in 22 minutes, the last one in 1 hour and 55 minutes, the average being 1 hour and 3 minutes. Records were taken of the time they continued to shoot. The shortest time was 1 hour and 20 minutes; the longest, five hours and two minutes; the average, 3 hours and 7 minutes.

In another experiment a canker was drenched with water in the woods and after it started to shoot, it continued for 2 hours and 35 minutes.

In a third experiment, a well infested small log was brought into the laboratory and sprayed with an atomizer. It began shooting in 44 minutes and was still shooting in places after 3 hours and 30 minutes. In this experiment it was noticed that, whenever the surface of the bark and pustules became dry, the spores ceased shooting. We may say then in general that as long after a rain on the surface of the bark remains wet, the spores will continue to shoot. This leads us to the next question.

Duration of shooting, if the bark remains wet.—On August 18th, five pieces of bark about 1 cm. square, were started to shooting in a moist chamber in the usual way. Records were taken three times a day of whether they were still shooting. One of them became covered with Pencillium and stopped shooting after six days. Of the others, one continued shooting 17 days, one 14 days, another 22 days and the last one, 25 days. Each of these, however, occasionally missed a day or part of a day. Later experiments gave similar results although no longer record than 25 days was ever obtained. Since it is not likely that a period of continuous rainy weather would be longer than 25 days, we may say in general, that the spores will continue to shoot as long as the bark is wet.

Effect of dessication on resumption of shooting.—On July 23rd, two pieces of bark were started to shooting spores in the regular way. After it was determined that they were shooting well, they were removed and thoroughly dried for a day, then tested again for shooting, after which they were dried for two days and tested, etc., being dessicated alternately for one and two days. For 27 days this experiment was continued, and on wetting each time, they continued shooting. The experiment was discontinued on the 19th of August because of contaminations.

In a similar experiment, allowing the bark to dry, however, one day between each test, spores were ejected on every test for 14 days. In a third experiment they were dried a week between each test. This experiment was in progress four weeks and at each test spores were ejected.

Distance to which spores will be ejected.—W. H. Rankin reports that they will be ejected with sufficient force to throw them 5 mm. straight upward. Numerous tests have been made in our laboratories. In general they will easily shoot from 4 to 7 mm. and often much higher. The highest record secured as yet is 22 mm. A more important question is: How far will they shoot horizontally? One is surprised in watching the course of the spores, by the "light beam" method to find that the majority follow a rather regular "sporabola," as Buller has named it; some of them seem to be lighter and float off further afield than the others. The following experiment will give some idea of their power to shoot horizontally when all air currents are excluded as far as possible. A piece of shooting bark about 5 mm. square was supported 1 inch above the edge of a sterile plate of agar, so that the ostioles pointed out horizontally over the plate. All this was done under a bell jar. After allowing the perithecia to shoot five minutes, the plate was covered and stored to see how the colonies of the fungus would show up. At the end of three days 508 colonies of Endothia appeared. From the region below the bark, diverging lines of closely crowded colonies appeared for 30 mm. Beyond this the colonies were irregularly scattered over the plate. The farthest one was 89 mm. from the point where the bark was placed.

These experiments show that without doubt, the spores are shot far enough into the air so that the wind will have abundant opportunity to catch them up and carry them to other trees.

Rate of ejection of spores.—The following experiment was carried out to determine the rate of shooting from a single ostiole. A piece of shooting bark was mounted on the stage of a microscope and a single ostiole found, from which spores were shot on to the slide. The spores on the slide were counted and the following data secured:

Slide Number.	Time of Exposure.	Perithecium A.	Perithecium B.
1,	30 seconds,	212	127
2,	30 seconds,	109	118
3,	30 seconds,	103	63

This gives us the further data:

The greatest number of spores per second,	7.1
The smallest number of spores per second,	2.1
Average number of spores per second,	4.06

Using the average as the basis of our calculations, this would give us 14,000 spores per hour or at the rate of 345,600 per day for one ostiole. By watching the ostioles under the microscope each discharge can be noted by the breaking of the film of water over the ostiole. It has been determined that with each discharge 8 spores are ejected. Taking the average in the table above, this would give us one discharge for every two seconds.

Another experiment which will give an idea of the rate of discharge was as follows: A canker on a small trunk was drenched with water and as soon as it began discharging spores a 10 cm. plate of sterile agar was exposed horizontally under it for five minutes. Nine thousand seven hundred and thirty-three colonies developed on the plate. The actual number of spores, of course, would be much greater than this.

Length of time required for germination of ascospores.—In favorable weather, ascospores will begin to germinate usually within five hours after ejection. The shortest record obtained in a long series of tests was 1 hour and 25 minutes. At most a spore would have to be kept moist only a few hours to become established in a new tree.

As to the mechanics of the process of ejection, very little is known and nothing has been done as yet in our laboratories. That it is connected with the life of the organism and not a mere physical process, is indicated by the fact that when the spores are killed by a treatment of four minutes with formaldehyde gas, they are no longer ejected from the perithecia.

Spore content of the air.—To determine whether the spores of the blight fungus were really floating about in the air two methods were used. Both of these methods were first used in badly infested tracts during dry weather, i. e. while the trunks of the trees were dry. Over one hundred plates were exposed and 500 liters of air were tested with the aspirator but since not a spore of Endothia was detected, it was decided that if there were any in the air in dry weather, they could not be detected by the methods used. Since it had previously been determined that the spores are ejected only after rains, all other tests were made while the trunks of the trees were wet. Since, however, it was inconvenient to have to wait for rains, the trunks were usually drenched with water by hand.

The aspirator method was the first one tried. A 15 liter flask was filled with water and a sterile sugar tube put in the opening at the top. The water was then permitted to run out slowly through a faucet at the bottom. The water in the bottle being replaced by air, which passes through the sterile sugar in the tube, any spores that were in the air, would be retained by the sugar. The sugar was then plated out and the number of spores per liter of air calculated from the number of colonies that developed on the plates and the number of liters of air drawn through the tube. The result of a number of tests made in this way are given in Table V. In general, this method was not found so satisfactory as the next one described, but was more accurate in giving more exact figures as to the number of spores per unit quantity of air.

TABLE V.

Showing the number of Spores contained in the air as determined by the aspirator method.

Number of liters of air.	Distance out from canker.	Distance below the canker.	Number of spores detected.
30,	2 ft. and 5 in.,	......	20
30,	1 ft. and 11 in.,	......	12
8,	4 ft.	......	......
15,	2 ft.	......	10
15,	6 in.,	......	33
15,	5 in.,	......	......
15,	2 in.,	......	40
15,	6 in.,	6 in.,	......
15,	......	22 ft.	50
15,	......	14 in.,	250
15,	......	6 in.,	110
15,	1 ft.	1 ft	75
15,	2 ft.	2 ft.	85
15,	1 ft.	1 ft.	60
15,	5 ft.	5 ft.	180
15,	3 in.,	5 ft.	210

Summary: Two hundred and sixty-three liters of air taken from distances varying from two inches to two feet out from and from six inches to twenty-two feet below the canker gave a total of 1135 spores, an average of 4.3 spores per liter.

The second method was as follows: Badly infested trunks of small trees were brought into an open place near the laboratory and induced to shoot spores by drenching with water. Sterile plates of chestnut bark agar were exposed for varying lengths of time, mostly so that the wind blew from the cankers to the plates and at various distances. The number of colonies of Endothia was counted, usually after three days. If there was any doubt about the identity of a colony, it was transferred to agar slants until identified by further growth. By this more convenient method, we were able to catch spores at a distance of more than 50 feet to the windward from the logs, but never more than a few inches against the wind. No effort was made to catch them at greater distances than 51 feet, but since they could easily be detected at that distance in a moderate wind and on a level with the canker, it would not be hard to imagine them carried for miles if they were on mountains, as we have often found them, and with a strong wind blowing. The results of a series of exposures is given in Table VI.

TABLE VI.

Showing the results of exposure of sterile agar plates near infested trees. Plates exposed so that the wind blew from the infested trees toward the plates.

Number of plates.	Distance from log.	Distance below canker.	Time exposed.	Plates discarded.	Average colonies per plate.
16,	1 inch	....	3-5 min.	4	3932
15,	1 inch	2-4 inches	3-5 min.	....	466
13,	1 inch	6-36 inches	3-25 min.	7	78
23,	2-6 inch	....	2-30 min.	7	890
40,	6-24 inch	....	3-30 min.	21	23
16,	2-36 inch	6-36 inches	15-60 min.	9	51
51,	3-9 ft.	....	10-80 min.	46	3
85,	9-30 ft.	....	20-60 min.	77	5
46,	30-51 ft.	....	60-90 min.	44	5

Exposed to side and Back of the Trees.

	Back of log.	To side of log.			
13,	....	12-90 inches.	10-25 min.	3	....
14,	1-26 in.	....	5-60 min.	7	4

A similar series of exposures with only 12 plates, however, at distances of only a few inches was tried with logs on which were numerous conidial tendrils but the results were entirely negative.

Inoculations by wind-borne spores.—Having demonstrated then that the spores are carried in great abundance by the wind, the next thing to be demonstrated was that these spores, falling into a wound in that condition, could produce infection. In order to duplicate more nearly natural conditions the following plan was followed in making the inoculations: Various kinds of wounds were made in the bark of healthy trees. Then ascospore bark taken from cankers and which had been determined to be shooting spores was suspended so that the spores were ejected toward the wound in the healthy tree. There was no way for the spores to get from the diseased bark to the healthy tree, except to pass through the air. After exposing it for a time, as given in the table, the wound was covered with cotton to prevent spores entering from any other source. The wounds were always made with a sterilized instrument and the bark was previously sterilized by washing with mercuric chloride or formalin. That this method was entirely successful is demonstrated by the results given in Table VII.

TABLE VII.

Showing results of inoculating with naturally ejected ascospores.

Kind of wound.	Distance of bark from wound.	Length of exposure.	Number of inoculations.	Number successful.
V-cut,	¼ inch,	15 min.	85	63
V-cut,	1 inch,	1½ hrs.	313	236
V-cut,	½ inch,	15 min.	22	12
V-cut,	1 inch,	1 hr.	391	370
V-cut,	¼ inch,	1 hr.	152	135
Broken branches,	½ inch,	45 min.	138	25
Tangential slice,	¼ inch,	1 hr.	28	27
Bruises from stone,	¼ inch,	2 hrs.	49	49
V-cut,	¼ inch,	1 min.	59	47
Longitudinal slit,	¼ inch,	1 hr.	12	2
Jagged knife cut,	¼ inch,	2½ min	62	55
V-cut,	¼ inch,	1 hr.	65	59
V-cut,	1 inch,	2 hrs.	19	19

In another series of inoculations the ascospore bark was placed at a greater distance from the wound and a draft created toward the tree by a hand bellows. These inoculations were also successful as indicated by Table VIII.

TABLE VIII.

Showing results of bellows inoculations.

Kind of wound.	Distance of bark from wound.	Length of exposure.	Number of inoculations.	Number successful.
V-cut,	3-12 in.	10 min.	19	15
V-cut,	1-4 ft.	10 min.	38	16
V-cut,	1-2½ ft.	15 min.	128	65

Another method of inoculating with dry ascospores was to crush the ascospore stromata in a mortar and then blow the fine dust into the air and let it settle down into wounds which had been made in healthy trees. The results of this series are given in Table IX.

TABLE IX.

Showing results of inoculations made by blowing crushed ascospore stromata into the air.

Character of Wound.	Number of inoculations.	Number successful.
Jagged hole,	230	47
Shot hole,	19	14
Breaking off dead limbs,	120	7
Hitting tree with blunt axe,	100	72
Jagged knife cut,	200	64

Check wounds were made in all the three preceding series but no cankers developed about them.

Another experiment to demonstrate the agency of the wind in carrying the spores was carried out as follows: Clumps of coppice growth chestnut were selected in each one of which was one or more trees with ascospore-bearing cankers. Wounds were made on the trees surrounding the cankered one, these wounds facing the cankers of the diseased tree. Sterile implements were used in making the wounds and the bark was previously sterilized for 25 minutes with mercuric chloride. These wounds were then covered with fine meshed wire (50 meshes to the inch) which was tacked down with a layer of cotton at the edge to insure it against the entrace of insects. A piece of cotton was tied very tightly just above the wired area to insure against any spores being washed down from above. The intention of this experiment was to exclude every possible agency for transport of spores except the wind. The cankered trees were drenched with water once a day for ten days. The wounds were at a distance of from one to five feet from the cankers. Of the 559 wounds made and protected in this way, 114 had developed cankers when the screens were removed at the end of three months. The wounds which were facing the central canker showed the greatest per cent. of infection. This is undoubtedly the most convincing of all the inoculation experiments with wind-borne ascospores.

Summary. The following demonstrated facts then lead us to believe that the wind is an important factor in the spread of the disease.

1. Mature ascospores can be found at any time of the year.
2. After every rain these are thrown out into the air in countless millions.
3. They are readily carried about by the wind.
4. Dry ascospores thus carried produced a high per cent. of infection in almost any kind of a wound.
5. Wounds are very common on chestnut trees.

LONGEVITY OF THE SPORES.

In studying the methods of dissemination of the fungus it is important to know how long the spores will retain their power to germinate and produce new infections. During the summer the writers began three sets of experiments to answer the three following questions: How long will ascospores retain their vitality after being ejected from the perithecia? How long, if they remain dry in the perithecia? How long will the conidia retain their vitality? None of these have been completely answered yet but the results up to date are given below.

1. **Ascopores after ejection.**—Clean slides were suspended over active ostioles and when clumps of ascospores had been deposited on them, they were stored in boxes in the laboratory to be tested at intervals for germination. The tests were made by covering the clump of spores with a drop of water and keeping the slide in a moist chamber over night. On the opposite end of each slide was placed a drop of water containing fresh ascospores to serve as a check. The percentages of germination were counted on the following day. As far as possible all slides for each series were secured from the same pustules. The first tests were made as soon as the spores were ejected and the percentages thus obtained may be considered as additional checks on the later tests. The results of two series are here given, the first being at Charter Oak and the second at Mt. Gretna:

Charter Oak.

At time of ejection (July 11),	test 90%	check 90%
After four weeks,	test 15%	check 85%
After six weeks,	test 10%	check 95%
After eight weeks,	test 10%	check 87%
After thirteen weeks,	test .03%	check 89%

Mt. Gretna.

At time of ejection (August 2nd),	test 95%	check 95%
After two weeks,	test 95%	check 95%
After four weeks,	test 50%	check 97%
After seven weeks,	test 25%	check 82%
After seventeen weeks,	test 14%	check 81%

2. **Ascospores in the perithecia.**—Bark containing mature ascospores was stored in open boxes in the laboratory. The results of three series, in which the same technique as in the preceding series was employed, are as follows:

Ascospores from bark collected at Charter Oak, June 25th, germinated to the extent of 40 per cent. after fifteen weeks (October 8th). Checks gave 95 per cent. germination.

Ascospores from bark collected at Mt. Gretna on June 28th, germinated to the extent of 65 per cent. after twenty-three weeks (December 6th). Checks gave 83 per cent. germination.

Ascospores from bark collected at Charter Oak May 10th germinated to the extent of 64 per cent. after 29 weeks (December 3rd). Checks gave 69 per cent. germination.

3. **Conidia.**—To test the longevity of conidia kept dry an experiment was carried out as follows: Spore horns were collected from trees in the woods on June 27th and stored in the laboratory in vials with cheese cloth tied over the tops. On July 25th they gave a fair per cent. of germination in rain water acidified with sulphuric acid. Since, however, this method was not reliable and since they could not be germinated in ordinary water, the next test, four weeks later, was by making streaks on agar slants. Six streaks gave successful cultures. On September 13th (11 weeks) they were tested by placing bits of the spore horns on sterile twigs in test tubes. Five inoculations made in this way gave just as good cultures as the checks made with fresh conidia. Similar results were obtained by this method at the end of 15 weeks and again at the end of 19 weeks. There is no doubt then that conidia can produce infection after being kept dry in the spore horn stage for 19 weeks.

All of these experiments are still in progress and much longer records are anticipated.

INOCULATION AND GROWTH EXPERIMENTS

Many important questions in regard to the life history of *Endothia parasitica* have never been answered. Too many statements have been based on mere casual observations on natural cankers. We have very little actual data on the rate of growth for various months of the year, how soon after inoculation the pycnidia appear, when the perithecia develop, what parts of the host can be infected, etc. Several thousand inoculations have been made at different times and under different conditions to settle some of these points.

RATE OF GROWTH OF THE CANKERS PER MONTH.

After inoculation the canker usually begins to show in two weeks if the inoculation is made with canker tissue or with mycelium from a culture. If, however, it is made with ascospores or conidia it shows very little, until from three to five weeks. Often no growth seems to take place for several months, then it suddenly begins to grow. Such cases are the exception, however, and not the rule. After the inoculation had been made from two weeks to a month, depending on the method, a white line was painted around the edge. At the end of each succeeding month, the canker was again outlined. Thus at the end of the year we will have a complete monthly record of the increase in the size of the cankers. The growth up and down the tree is more rapid than that around the tree so that, no matter how the inoculation is made, the canker soon becomes oval in shape. The rate of growth up and down the tree also varies greatly,—much more than that around the trunk. It seems to depend a great deal on the nature of the wound and the condition of the tree. This factor, however, is not so important. What we wish to know is: How fast does it grow around the tree? It is the girdling that kills and not the longitudinal growth. Up to the present we have the records for six months. These are given in Table X.

TABLE X.

Showing the monthly rate of growth of cankers in summer of 1912. Using transverse diameter of the cankers.

Month.	Number of cankers.	Average growth per month in centimeters.
June,	31	1.887
July,	200	2.779
August,	186	2.834
September,	140	1.846
October,	53	1.92
November,	93	---------

These are averages for a large number of cankers. Individual cases showed growth sometimes more than twice as great, but in general, the variation was slight. It will be noticed that the greatest growth was during the months of July and August, which were very warm months with an abundance of rainfall, while September and June were much cooler. There is an impression abroad that a canker will girdle a large tree in one year. A table is given showing the length of time it would take for a canker, growing all the time at the rate they did in August of this year, to girdle a tree of a given diameter (Table XI). However, it is very doubtful if the cankers will grow at this rate during the winter, so that it is likely that it would take much longer than this.

TABLE XI.

Showing the time it would take a canker to girdle a tree at the rate they grew in August, 1912

Diameter of tree.	Time required to girdle tree.
1 inch,	2 months and 12 days.
2 inches,	5 months and 19 days.
3 inches,	8 months and 12 days.
4 inches,	11 months and 9 days.
8 inches,	22 months and 15 days.
12 inches,	34 months and 0 days.

TIME OF APPEARANCE OF THE FRUITING STAGES.

There has been much dispute as to whether the winter or ascospore stage was developed on cankers during the first year, also as to the time it takes for the two stages to appear. Records have been and are being kept on over two thousand cankers to determine these points. The records of average plots which have completed the cycle, are given in Table XII.

TABLE XII.

Showing the stages of development of the fungus during the summer of 1912. From artificial inoculations.

Plot.	Date of inoculation.	Method.	Number of inoculations.	Appearance of spore horns.	Appearance of perithecia
4,	May 29,	Diseased tissue in slit,	31	July 11,	Oct. 3
8,	June 9,	Diseased tissue in slit,	76	July 5,	Sept. 3
10,	June 9,	Ascospores in stab,	184	July 23,	Oct. 1
12,	June 11,	Conidia in stab,	96	(No data)	Oct. 2
23,	June 26,	Diseased tissue in slit,	35	July 25,	Sept. 5
27,	June 27,	Diseased tissue in slit,	47	(No data)	Oct. 3
33,	July 12,	Diseased tissue in slit,	20	Sept. 6,	Oct. 4

In general it may be said that under natural conditions in the summer time the spore horns will be developed in from three to six weeks, and that the winter or ascospore stage will develop in ten weeks or more. The fact that the perithecial stage on all these plots appeared in September and October should not be interpreted as indicating that the approach of winter had any influence in bringing about this stage. There has been no time during the summer when developing perithecia could not be found in some localities. In cultures on agar the conidia are produced more quickly. On potato agar, they are almost always developed in less than two weeks from conidial streaks. They have been developed in six days from ascospores caught on chestnut-agar plates after being naturally ejected from the perithecia.

COMPARATIVE GROWTH OF THE FUNGUS ON YOUNG AND OLD BARK.

In order to determine whether the fungus grows as rapidly on the heavy barked-trunks, as on the thinner-barked younger trees and branches, twenty inoculations were made in old trees with heavy rough bark. At the same time, 78 inoculations were made close by in thin-barked trees. Since the bark on the old trees did not show any depressions where the cankers were, they could not be outlined, and the monthly growth of the two plots compared. Therefore at the end of twelve weeks the bark was peeled from the cankers on the old trees and the cankers measured on the cambium. It was noticed, however, that the fungus spread somewhat more rapidly in the bark of the heavy barked trees than in the cambium, so the figures are a little less than the real dimensions of the cankers. The average for the twenty cankers was 13.22 x5.58 cm. The average for the 78 cankers on the thin barked trees was 14.3x9.7 cm. According to these figures the growth is a little more rapid on the thin barked trees.

GROWTH OF THE FUNGUS ON LEAVES AND BURS.

Up to the present, it has never been found growing on either of these, and all attempts to inoculate green leaves and green burs have been unsuccessful. Dead burs and dead leaves, however, in moist chambers have been sucessfuly inoculated as given under the heading, "Man as the Disseminator." Seasoned dead wood was also inoculated and the fungus successfully grown on it.

GROWTH ON THE ROOTS OF THE CHESTNUTS.

That it will grow on exposed roots just the same as on the bases of the trees is a matter of common observation. Inoculations made on exposed roots were just as successful as those on the trunks. To determine whether it would also grow on subterranean roots 18 inoculations were made on June 27th, and the roots again covered with earth. The parasite appeared to grow in all cases, but did not produce typical cankers. The invaded areas became soggy and the growth was apparently very slow. Isolations from the soggy areas however, gave pure cultures of Endothia.

GROWTH ON THE GREEN SHOOTS OF THE CURRENT YEAR.

Murrill (1) does not believe that the shoots of the first year become infected. So far as the literature shows, no one has ever found blight on them, or successfully inoculated them. The following experiments give the results obtained at Charted Oak on this point.

Experiment: Inoculation of sterilized first year twigs. Fifteen fresh pieces of first year twigs were sterilized in test tubes by washing in a 0.5 solution of mercuric chloride and inoculated as follows:

Five with conidia. Four were successful.

Four with diseased bark. Three were successful.

Six with agar culture. All were successful.

The growth on all of these was characteristic for Endothia and differed very little from cultures on older twigs under the same conditions. This proved that failures to produce infection of first year twigs were not due to any injurious substance in the twigs themselves.

Experiment: Inoculations of first year sprouts in the woods. The methods of inoculation and the results are given in Table XIII. The cankers produced were typical in every way. Some of the sprouts had already died from the cankers when the plot was destroyed. This proves beyond question that cankers can be produced on first year twigs, but offers no explanation of why they are so rarely found there in nature.

TABLE XIII.

Showing results of inoculation in first year shoots.

Date.	Plot.	Method of inoculation.	Number of inoculations.	Percentage successful.
July 27,	48	Ascospores in slit protected with cotton,	25	4.0
July 27,	48	Ascospores in slit not protected with cotton,	24	37.5
July 27,	48	Diseased tissue in slit protected with cotton,	13	100.0
July 27,	48	Diseased tissue in slit not protected with cotton,	6	83.3

COMPARISON OF SUSCEPTIBILITY OF TREES IN THE OPEN AND IN DENSE WOODS.

One would naturally expect that trees in dense woods would be more easily attacked on account of better moisture conditions. Plots were therefore selected in dense coppice and check plots in open places, where the trees were trimmed high and were far apart and no underbrush around them. Here they would have plenty of opportunity for air currents and abundance of sunlight. These were inoculated on the same day. The results are given in Table XIV. This summer has been very moist and the results might be different on an average year, but certainly the results here do not argue for much advantage of either location over the other. One fact however was observed in this series of inoculations which is worthy of notice. Where the inoculation wound is made in a tree in the open, a callus begins to form at once and for several weeks one is tempted to believe that the tree has succeeded in excluding the invader. There is evidently a continued struggle between the host and the parasite and if we were dealing with a less virulent parasite, the struggle would undoubtedly result in favor of the former. But the fungus gradually works in under the callus and soon becomes too strong to be resisted. On the other hand it is very rarely that a callus is formed where the tree is in dense woods.

TABLE XIV.

Showing the difference in susceptibility of trees in the open and trees in dense woods.

Open Woods.

Date.	Plot.	Method of inoculation.	Number of inoculations.	Percentage successful.
June 28,	31	Mycelium from culture in slit,	35	88.5
Aug. 5,	50	Conidia in water in V-cuts,	56	76.8
Aug. 5,	50	Ascospores in water in V-cuts,	72	55.5
Aug, 15,	53	Ascospores in water in V-cuts,	36	44.4
Aug. 15,	53	Diseased tissue in slits,	42	100.0

Dense Coppice.

Date.	Plot.	Method of inoculation.	Number of inoculations.	Percentage successful.
June 27,	29	Mycelium from culture in slit,	40	75.0
June 27,	29	Diseased tissue in slit,	40	100.0
Aug. 5,	51	Conidia in water in V-cuts,	30	50.00
Aug. 5,	51	Ascospores in water in V-cuts,	30	90.0

THE EFFECT OF ALTITUDE AND WATER CONTENT OF THE SOIL ON THE GROWTH OF THE FUNGUS.

Some observers have noticed that the blighted trees are always on low land; others have observed that they are always on the side of the slope; others that they are always up high on dry ground. The theory that the water content of the tree determines its susceptibility, has also been advocated. The laboratory and experimental plots at Charter Oak are admirably located to test out these theories. Along Shavers Creek, below the laboratory, there are marshy places where the roots of the chestnut trees have not been out of the water all summer. The woods is dense and conditions could not be invented where the water content of the trees would be higher. Back of the laboratory, Lead Ridge, a dry rock ridge of Tussey Mountains, rises about 1200 feet above Shavers Creek and is covered with chestnut trees. They grow under very dry conditions at the summit and there are all intermediate conditions on the slopes. Plots were inoculated under all these conditions. The methods and results are given in Table XV.

The results of the experiment indicate that the altitude and soil drainage have very little to do with the susceptibility of the host or rate of growth of the fungus.

TABLE XV.

Showing the effect of moisture and altitude on the growth of the fungus.

Plot.	Date of inoculation.	Number of inoculations.	Location.	Percentage infection.	Growth per month.		
					July.	August.	September.
29*	June 27, ------	40	Summit of dry ridge 1200 ft.,	100.0	1.66	2.58	2.14
29†	June 27, ------	40	Summit of dry ridge 1200 ft.,	82.5	1.55	2.28	1.82
29‡	June 7, ------	54	Half way up the ridge, ------	96.3	2.66	3.10	2.78
16*	June 18, ------	80	Half way up the ridge, ------	86.3	2.55	2.69	2.78
16†	June 18, ------	44	Half way up the ridge, ------	95.5	2.21	2.66	2.90
19*	June 21, ------	32	Marsh near creek, ------------	93.7	2.21	3.10	2.46

*Inoculations made by placing canker tissue in longitudinal slit.
†Inoculations made by placing mycelium from culture (No. 31 Charter Oak) in longitudinal slit.
‡Inoculations made by placing mycelium from culture (No. 32 Mt. Gretna) in longitudinal slit.

ENDOTHIA PARASITICA ON OTHER HOSTS.

NATURAL OCCURRENCE.

This fungus is known to cause a serious disease only on chestnut. During last summer, however, a fungus which was in all outward appearance the same, has been collected and sent to us or has been found by the writers on the following hosts:

Quercus velutina (Black Oak).
Quercus alba (White Oak).
Quercus prinus (Chestnut Oak).
Rhus typhina (Staghorn Sumac).
Acer rubrum (Red Maple).
Carya ovata (Shag-bark Hickory).

The fungus was isolated from all of these except *Quercus prinus* They were cultured on various media and as far as their culture characters are concerned, they cannot be distinguished from the regular *Endothia parasitica,* on chestnut.

In most of the cases where it was on other hosts it was growing as a saprophyte, seeming to prefer fire-scorched or lightning-killed trees. In two cases, however, on the white oak, it had all the appearance of a parasite, plainly pushing out into the living tissue. So many of these specimens were sent in and there was such general interest in them, that it was decided to run a set of experiments to determine whether the fungus was the same on all these hosts, whether it was the same as that on the chestnut, whether they would infect chestnut and whether the regular Endothia would infect the hosts from which each was isolated. These experiments and the results are given below.

GROWTH ON STERILIZED TWIGS OF VARIOUS SPECIES.

Experiment: To determine whether the chestnut blight fungus would grow on sterilized twigs of other species than chestnut. Twigs of the following species were used:

Castanea dentata (Chestnut).
Quercus alba (White Oak).
Quercus prinus (Chestnut Oak).
Quercus macrocarpa (Burr Oak).
Quercus velutina (Black Oak).
Quercus rubra (Red Oak).
Quercus coccinea (Scarlet Oak).
Rhus typhina (Staghorn Sumac).
Nyssa sylvatica (Sour Gum).
Acer rubrum (Red Maple).
Liriodendron tulipifera (Yellow Poplar).
Juglans nigra (Black Walnut).
Carya ovata (Shagbark Hickory).

Pieces of these twigs, about three inches long, were put in test tubes with wet cotton in the bottom, plugged and steam sterilized. Six tubes of each species were used, two inoculated with conidia, two with ascospores, and two with mycelium from culture. The fungus grew on all of them and also produced pycnidia regardless of how they were inoculated. The growth on all the oaks, on sour gum and on sumac was just as rapid and as vigorous as on the chestnut twigs. On the others, however, the growth was much slower and not so luxuriant. This experiment was duplicated in two laboratories with the same results. Twigs of other species were not tried, but in all probability it would grow on other twigs besides those mentioned.

INOCULATIONS ON CHESTNUT WITH STRAINS FROM OTHER HOSTS.

Experiment: To determine whether the strains isolated from other hosts would produce typical cankers on chestnut. The isolations were made in each case from the original host, either from spore horns or from the diseased tissue, which was transferred to potato agar. Pieces of this agar were then introduced into slits in the bark as in our regular inoculations. The strain from hickory has not been used since it was isolated too late in the season. The results of the inoculations are given in table XVI.

The cankers produced were in every way typical, and grew with a vigor and rapidity equal to that of the strains isolated from the chestnut. There is therefore, no doubt that these strains are the regular chestnut blight fungus.

TABLE XVI.

Showing results of inoculations on chestnut with strains of the fungus from other hosts.

Date.	Strain.	Host from which isolated.	Number of inoculations.	Percentage successful.
July 20,	5	Black Oak,	22	95.5
July 20,	6	White Oak,	27	85.2
July 20,	36	Sumac (Staghorn),	20	95.0
Aug. 14,	38	Red Maple,	16	75.0

INOCULATIONS WITH ENDOTHIA ON HOSTS OTHER THAN CHESTNUT.

Experiment: To determine whether *Endothia parasitica* can be inoculated into other hosts and made to produce typical cankers there. The mehods and results of these inoculations are given in Table XVII.

TABLE XVII

Showing the results of inoculation with *Endothia parasitica* on hosts other than chestnut.

Date.	Plot.	Host inoculated.	Method.	Number of inoculations.	Percentage successful.
June 10,	11	Chestnut oak,	Diseased tissue in slit,	34	41
June 27,	26	Red maple,	Diseased tissue in slit,	32	----------
June 28,	31	Black oak,	Mycelium from culture in slit,	20	----------
June 13,	14	White oak,	Ascospores in stab protected,	51	78.4
June 13,	A14	Sumac,	Ascospores in stab. Unprotected.	58	----------
July 12,	34	Sumac,	Diseased tissue in slit,	32	71.9
July 12,	35	Yellow poplar,	Diseased tissue in slit,	22	----------
July 19,	37	Chestnut oak,	Ascospores in V-cut,	38	----------
July 19,	37	Chestnut oak,	Conidia in V-cut,	30	----------
July 19,	37	Hickory,	Conidia in V-cut,	22	----------
July 19,	37	Hickory,	Ascospores in V-cut,	27	----------
July 19,	37	Hickory,	Mycelium from culture in slit,	16	----------
July 19,	37	Hickory,	Diseased tissue in slit,	12	100
July 19,	37	Scarlet oak,	Ascospores in V-cut,	18	----------
July 19,	37	Scarlet oak,	Conidia in V-cut,	12	----------
July 19,	37	Scarlet oak,	Diseased tissue in slit,	6	100
July 19,	37	Black oak,	Diseased tissue in slit,	12	100
July 19,	37	Black oak,	Conidia in V-cuts,	12	----------
July 19,	37	Black oak,	Ascospores in V-cuts,	12	----------
July 20,	40	White oak,	Mycelium from culture,	10	----------
July 20,	41	Black oak,	Mycelium from culture,	9	----------
July 20,	42	Sumac,	Mycelium from culture,	26	100

The figures representing the percentages of successful inoculation in this table do not accurately represent the results of the experiments. In no case were typical cankers produced as on the chestnut. The inoculation was judged to be successful, when the spore horns of the fungus were produced on the bark of the inoculated tree.. This in all cases did not mean that it was growing there as a parasite. A wound is always necessary in making an inoculation, and there is abundant opportunity for the fungus to grow as a saprophyte on the injured tissue about the wound. This condition was especially noticed in the case of the hickory, black oak and scarlet oak. The growth on the white oak and chestnut oak was nearest like that on the chestnut. The fan-shaped areas of mycelium were found plainly advancing into the healthy tissue and there was an abundance of spore horns. The outline of the cankers continued to advance slowly for from eight to ten weeks. After that the fungus was apparently holding its own, but ceased to advance. As yet no oak tree has been found killed by the fungus. The growth on the sumac is entirely different from that on chestnut or on oak. No fan-shaped areas were found, but an abundance of spore horns, and also superficial pycnidia were produced on the edges of the inoculation wounds. The rate of growth varied with the condition of the host. Where the host was apparently in poor condition the growth was very rapid. Two trees of this kind were killed during the summer.

The ability of this organism to live as a saprophyte on other hosts is well illustrated on the infested woodlot previously mentioned at Anderson, Pennsylvania. This mixed stand of chestnut and chestnut oak was cut in the early spring of 1912. When the writers inspected the tract the following October, the characteristic reddish, flattened pycnidia were found on the top of almost every stump, irrespective of whether it was chestnut or oak. Many of the dead tops of the trees also had an abundance of the pustules of the blight fungus on them.

SUMMARY.

The results of these experiments indicate that the fungus is a weak parasite on white oak, chestnut oak and sumac. It has not shown any parasitic tendencies on any of the other species tried. Its attacks on the other trees is of practical importance, only in that they may be the means of keeping the fungus over in a locality where the diseased chestnut has all been destroyed.

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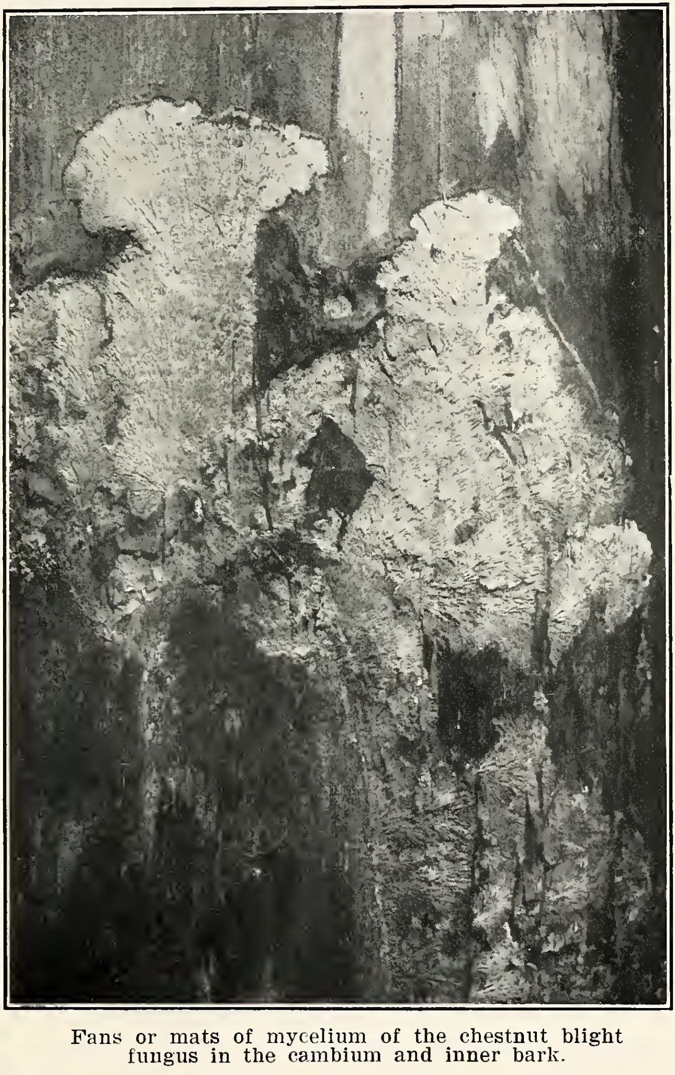
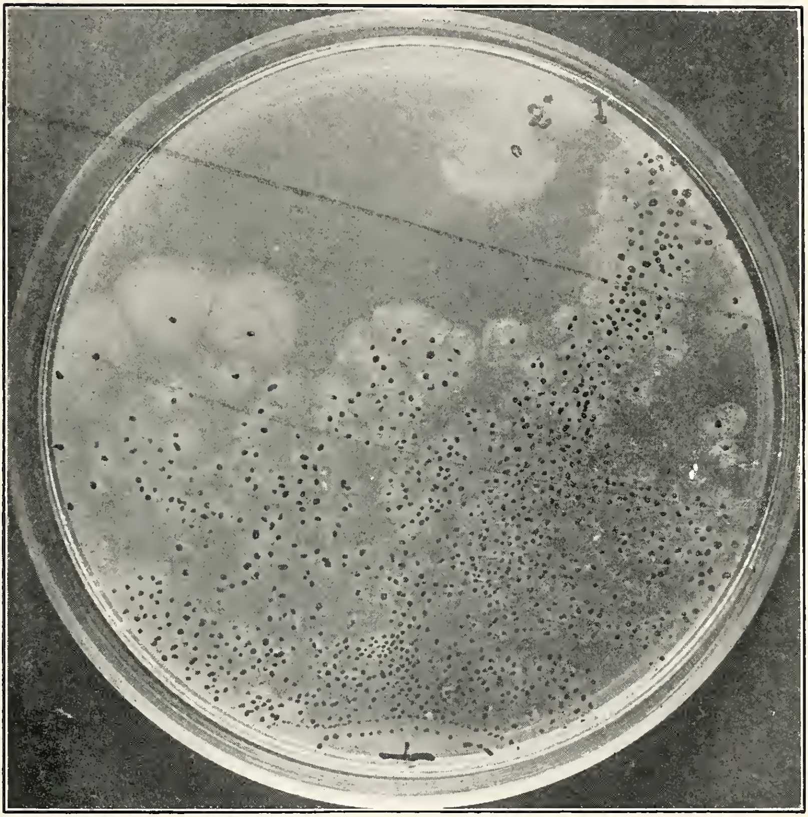
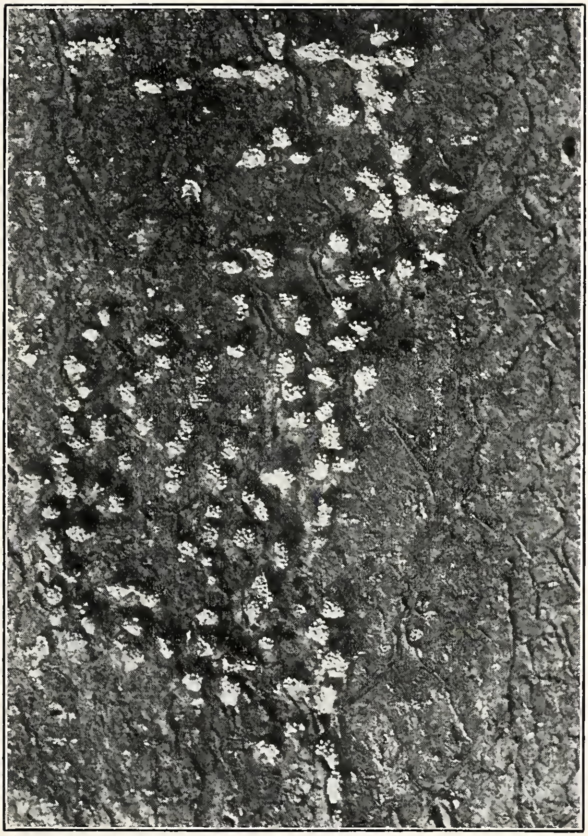


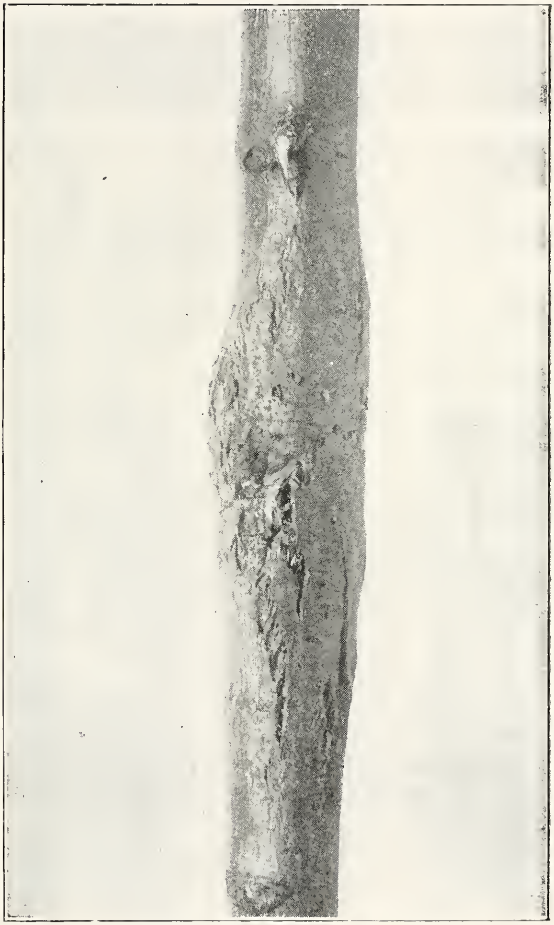
Fans or mats of mycelium of the chestnut blight fungus in the cambium and inner bark.



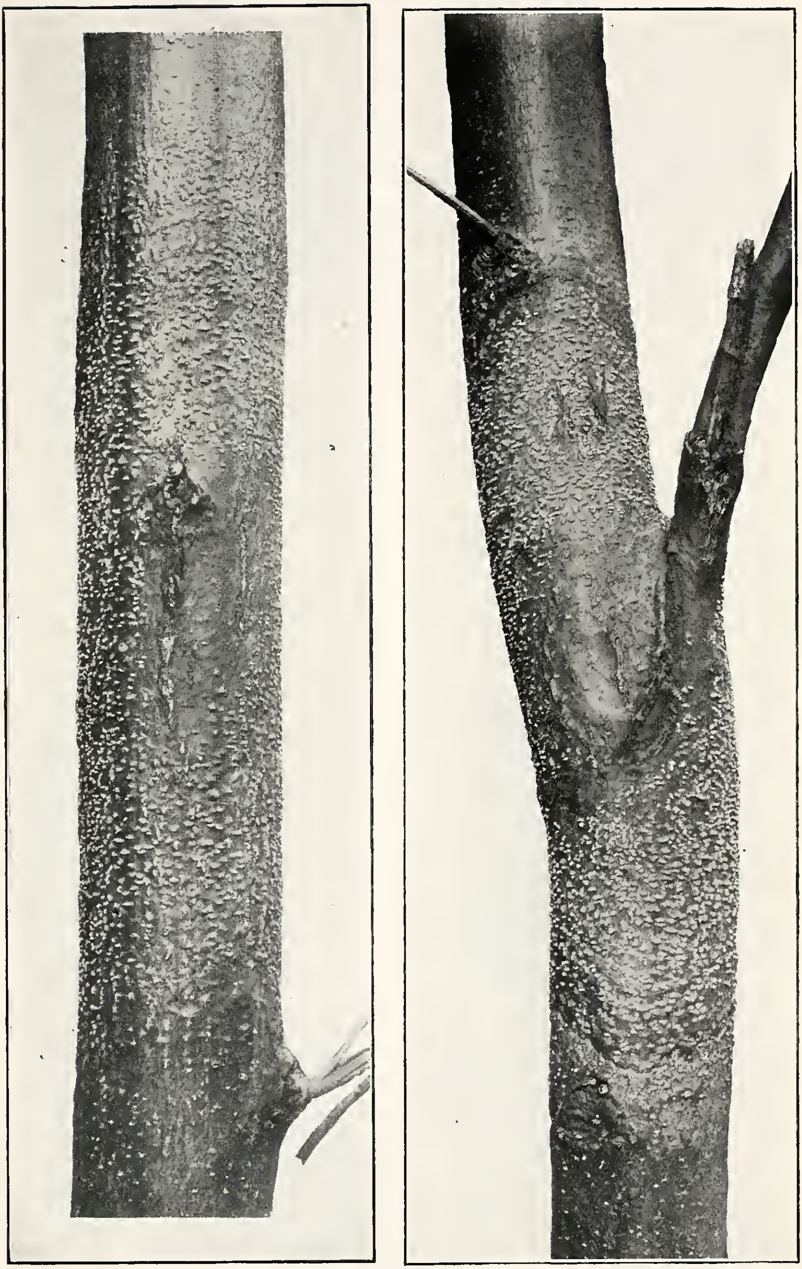
Colonies of the blight fungus on agar plate resulting from naturally ejected ascospores. Bark with active perithecia placed one inch above the plate over the dark line at bottom of plate. Each black point indicates one colony.



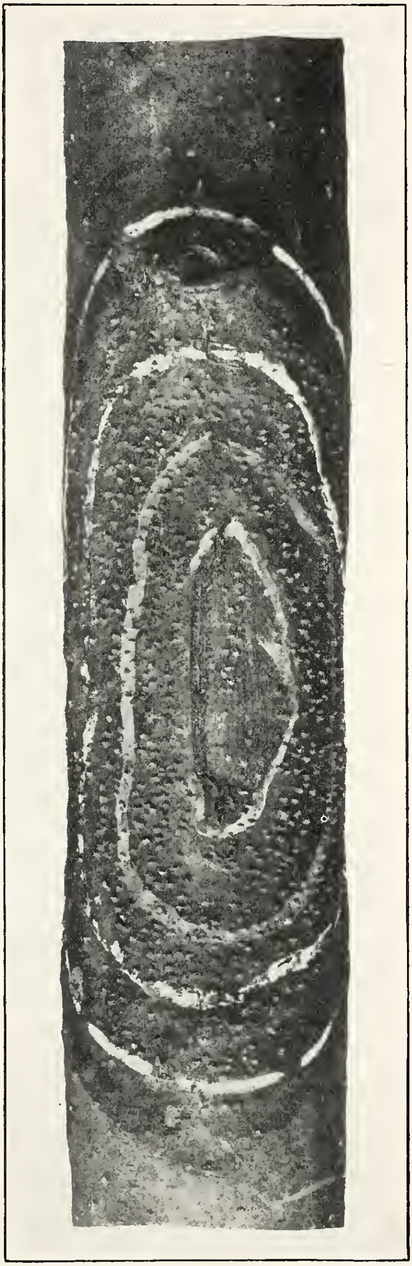
Stromata showing the necks of the perithecia.



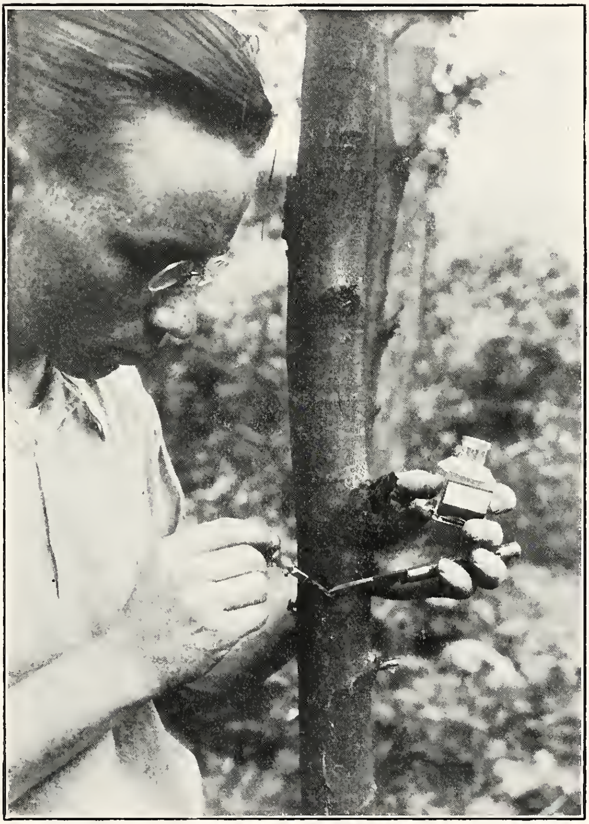
Hypertrophy type of canker.



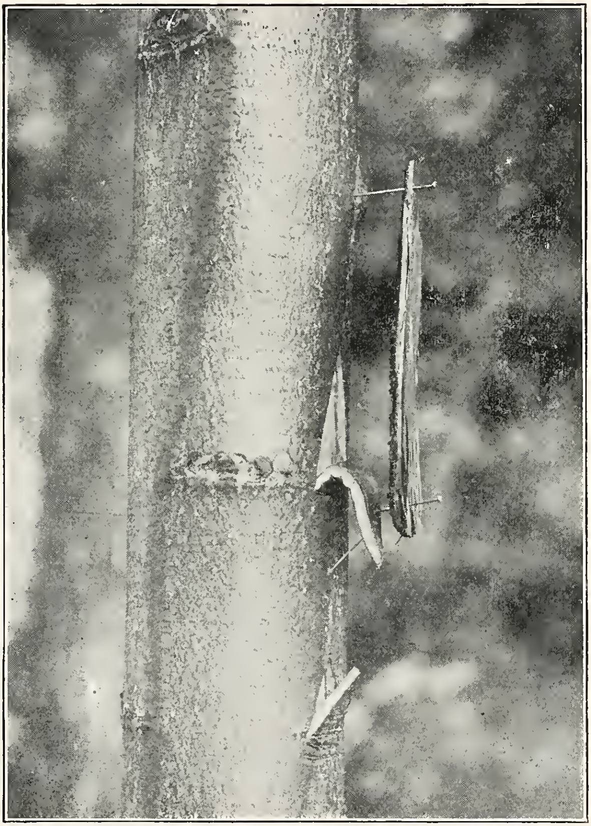
Cankers showing stromata.



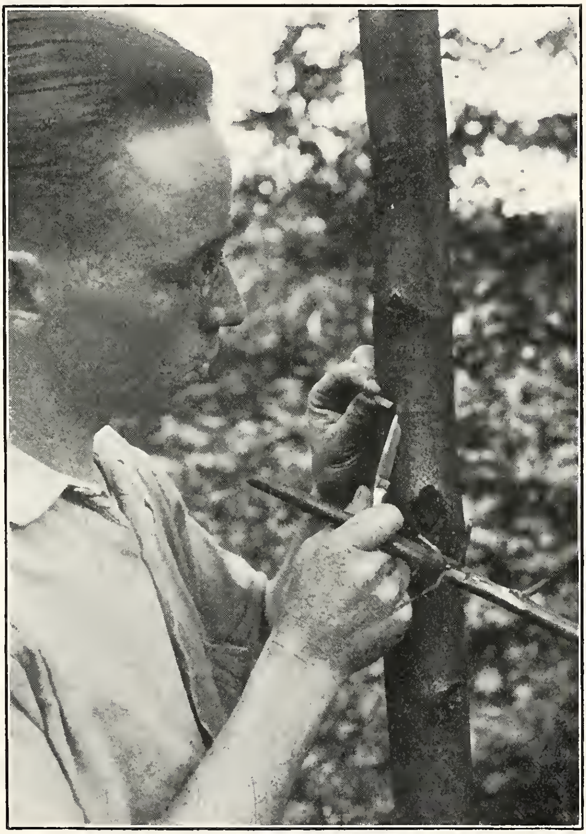
Painted outlines showing monthly growth of a canker, one-half natural size.



Inoculation with ascospores in stab in the bark.

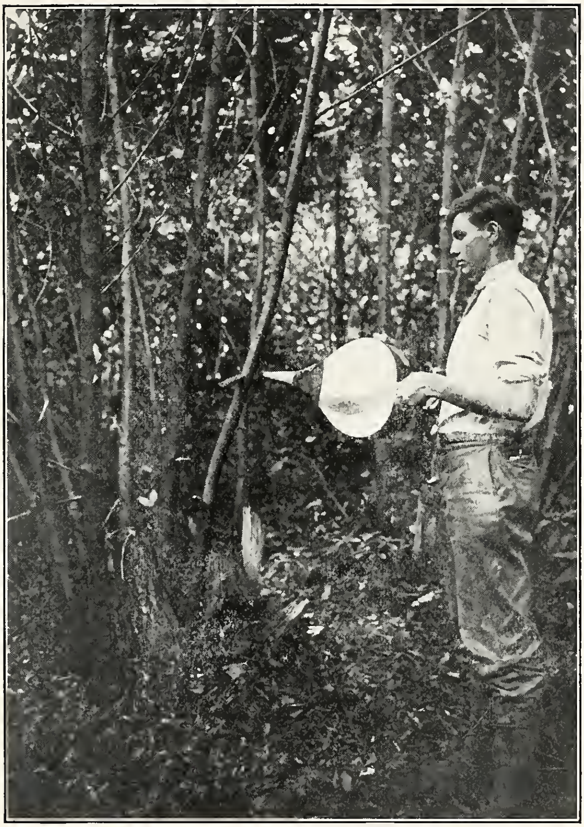


Inoculation with naturally ejected ascospores at close range.

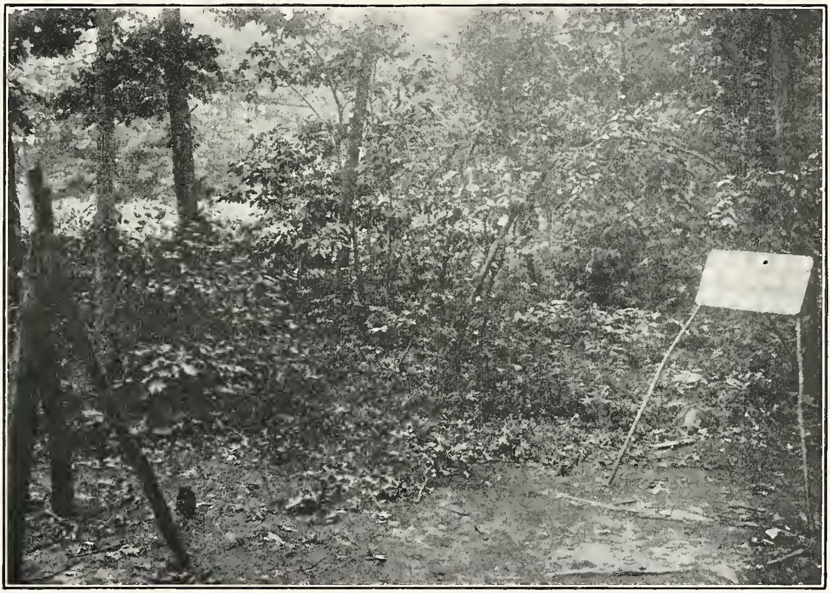


Inoculation with diseased bark.

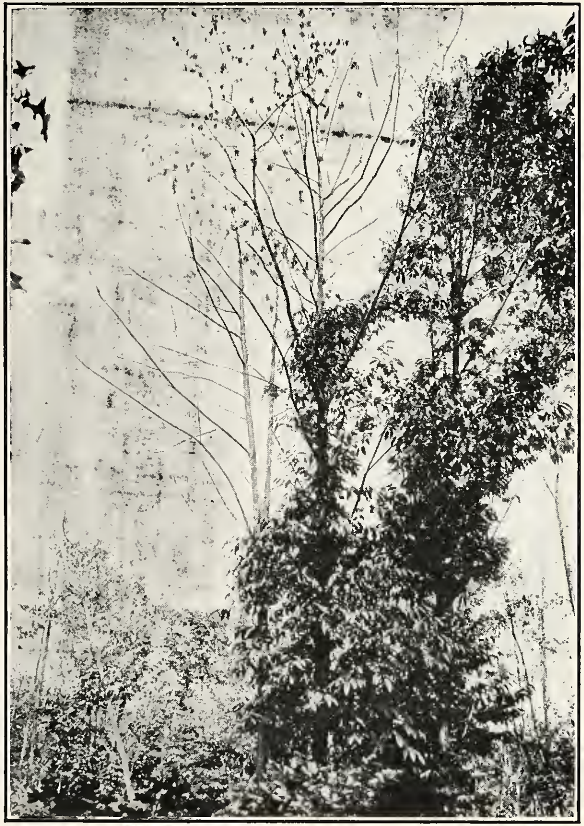
Inoculation at the base of broken twigs.



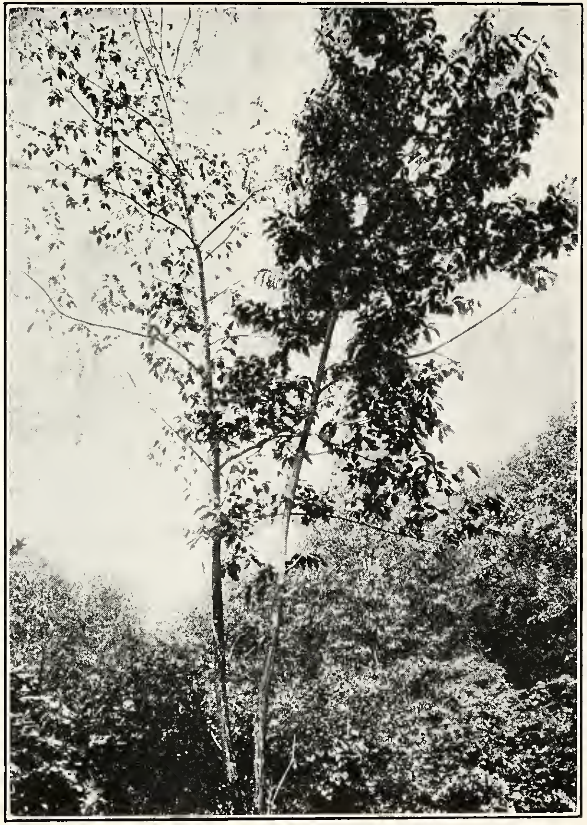
Artificial wind inoculation with bellows.



Testing the carrying power of the wind. Sterile plates exposed on tripod at the right. Active perithecia on the upright logs at the left. Wind blowing from the logs toward the plates.



Blighted and healthy chestnut tree in summer. Notice the small, undeveloped leaves on the diseased tree.



Blighted trees in summer, showing undeveloped leaves and water sprouts. The leaves came out in the Spring on the top of the tree in the centre, but remained undersized and died about the middle of the summer.

Logging experiment at Mt. Gretna, Lebanon Co., Pa.

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